Chapter 8

Dilute Acid and Autohydrolysis Pretreatment

Bin Yang and Charles E. Wyman

Summary

Exposure of cellulosic biomass to temperatures of about 120–210°C can remove most of the hemicellulose and produce cellulose-rich solids from which high glucose yields are possible with cellulase enzymes. Furthermore, the use of dilute sulfuric acid in this pretreatment operation can increase recovery of hemicellulose sugars substantially to about 85–95% of the maximum possible versus only about 65% if no acid is employed. The use of small-diameter tubes makes it possible to employ high solids concentrations similar to those preferred for commercial operations, with rapid heat-up, good temperature control, and accurate closure of material balances. Mixed reactors can be employed to pretreat larger amounts of biomass than possible in such small-diameter tubes, but solids concentrations are limited to about 15% or less to provide uniform temperatures. Pretreatment of large amounts of biomass at high solids concentrations is best carried out using direct steam injection and rapid pressure release, but closure of material balances in such "steam gun" devices is more difficult. Although flow of water alone or containing dilute acid is not practical commercially, such flow-through configurations provide valuable insight into biomass deconstruction kinetics not possible in the batch tubes, mixed reactors, or steam gun systems.

Key words: Dilute acid, Autohydrolysis, Pretreatment, Reactor, Lignocellulosic biomass, Batch, Flowthrough

1. Introduction

Pretreatment refers to the disruption of the naturally recalcitrant structure of lignocellulosic biomass to make cellulose and hemicellulose susceptible to an enzymatic hydrolysis step for generation of fermentable sugars. Over the years, various biological, chemical, and physical pretreatment technologies have been explored as ways to increase sugar yields, and several chemical technologies

Jonathan R. Mielenz (ed.), *Biofuels:* Methods and protocols, Methods in Molecular Biology, vol. 581
DOI 10.1007/978-1-60761-214-8_8 © Humana Press, a part of Springer Science+Business Media, LLC 2009

show great promise *(1)*. Judging the suitability of pretreatment options must take into account their impact on other steps with respect to such features as the sugar release patterns and solid concentrations for each pretreatment to ensure compatibility with the overall process, feedstock, enzymes, and organisms to be used.

Autohydrolysis occurs when biomass is pretreated with just steam and has been favored because of its long history of development and substantial industrial experience, which includes the use of large-scale equipment such as the batch Masonite gun used in the fiber board industry and the continuous screw fed STAKE II reactor *(2–4)*. However, hemicellulose sugar yields from autohydrolysis are limited to less than about 65% of the maximum possible, while adding dilute sulfuric and other acids can recover up to about 90% of the theoretical maximum. As early as 1898, sulfuric acid was employed to catalyze the hydrolysis of cellulose and hemicellulose in biomass to release sugars, although the costs were too high owing to the high concentration of the acid used and the low sugar yields *(5)*. Further developments lowered the concentration of sulfuric acid required, but a two-step thermochemical process was employed to accommodate the different temperature histories needed to obtain high yields of sugars from both cellulose and hemicellulose *(6)*. More recently, biological catalysis was substituted for the second thermochemical step to enhance glucose yields from cellulose *(7)*, but a pretreatment step was required, with removal of most of the hemicellulose prior to enzymatic hydrolysis of the cellulose in the solid residue being one of the first approaches. Not only did dilute acid achieve higher hemicellulose sugar yields than pretreatment with just water or steam in a batch or co-current flow mode, but it also produced a much higher ratio of monomeric to oligomeric sugars in the liquid *(7–16)*.

Pumping water or dilute sulfuric acid through the solids in a flow-through configuration produced better performance than dilute acid in a batch mode as measured by higher hemicellulose recovery, higher lignin removal, higher glucose recovery from cellulose, and less inhibitors than the conventional system *(17–24)*. However, high water and energy consumption and the difficulty in equipment development impede commercial applications of this method. Thus, the flow-through approach primarily has value for providing time release data that can enhance our understanding of hemicellulose hydrolysis, improve the technical foundation for biomass pretreatment, and lead to innovative, advanced pretreatment technologies. In this chapter, our focus is on the description of laboratory-scale equipment and methods that have been employed successfully for autohydrolysis and dilute acid pretreatments in batch and flow-through operation modes.

2. Materials

1. Batch tubular reactors are 0.5 in OD × 0.035 in. wall thickness stainless steel or Hastelloy C276 tubing (Maine Valve and Fitting Co., Bangor, ME). Reactors are heated by a 4-kW fluidized sand bath (model SBL-2D, Techne Co., Princeton, NJ) and monitored with a thermocouple probe (Omega CASS-18U-12, Omega Engineering Co., Stamford, CT).
2. The mixed batch reactor testing requires a 1-l cylindrical reactor made of Carpenter-20 or Hastelloy C (Parr Instruments, Moline, IL), which is rotated with an adjustable speed DC motor drive (A1750HC, Parr Instruments, Moline, IL).
3. The flow-through reactor parts can be purchased from Maine Valve and Fitting Co., Bangor, ME, or other supply houses to build a small tubular reactor (½ in. ID × 1.84 in. length) with an internal volume of 3.6 ml and a larger tubular reactor (½ in. ID × 6 in. length) with an internal volume of 14.3 ml. Flow-through reactions are monitored with a K-type thermo couple (Extech Instruments, 421501) and a pressure gauge (pressure range 0–1,500 psi, Cole-Parmer Instrument Co., Vernon Hill, IL) and a backpressure regulator (Maine Valve and Fitting Co., Bangor, ME). Temperature is monitored with a 0.25 in. stainless steel thermocouple (Omega CASS-18U-12, Omega Engineering Co., Stamford, CT).

3. Methods

3.1. Tubular Batch Pretreatment Reactors

Tubular pretreatments are generally carried out over a temperature range of approximately 140–180°C when dilute sulfuric acid is used, or from approximately 170 to 220°C when just water is employed. One key for batch pretreatment in tubular reactors is to ensure that the temperature is as uniform as possible across the tube diameter, and thermal analyses have shown that a tube diameter of less than 0.5 in. can meet this requirement (*25, 26*). Furthermore, because it is difficult to add biomass solids to very small diameter tubes, batch tubular reactors are often made from 0.5 in. OD × 0.035 in. wall thickness stainless steel or Hastelloy C276 tubing for water-only or dilute acid treatment, respectively. As shown in **Fig. 1**, the tubing is cut to 6 in. or other lengths compatible with the heating system and fitted with Swagelok couplings and removable threaded end caps. Although the total volume is about 14.3 ml, only about 6 ml of wet biomass solids is added to each to provide room for thermal expansion during

Fig. 1. Batch tube reactors and fluidized sand bath system.

heat-up. If the end caps are made from stainless steel to keep costs low, Teflon plugs must be installed at both ends of the tubing to protect them from the acid *(24, 27)*. A second key for small batch tubular reactors is the provision to rapidly heat up and cool down the reactor contents; the extended heat-up times for conventional electrical heaters and prolonged cool down with air do not generally meet this requirement. However, heated fluidized sand baths provide high heat transfer rates with safe fluid, and quenching by submerging in ice water is effective for rapid cool down.

For pretreatment with just water, biomass of the desired moisture content is just loaded into the tubes. For dilute acid systems, the biomass is first soaked overnight in a large volume of water of the targeted dilute acid concentration. Then, excess water is squeezed out until the desired moisture level is obtained prior to adding to the reaction tubes. A thermocouple probe is then inserted 3/16 in. and 2 in. deep in the center of the reactor to monitor the pretreatment temperature over the course of the reaction. Based on thermal modeling, a three-bath heat-up procedure *(28)* is used to minimize the effect of thermal transients in batch tubular reactors. First, the reactor is preheated to 100°C in boiling water for 2 min, and it is then immediately transferred to a sand bath held at 20°C above the target reaction temperature for 1 min. Next, the reactor is moved to a second sand bath controlled at the target reaction temperature at a reaction time defined as zero. After the target time is passed, the reactor is quickly transferred to an ice water bath and held there for 5 min to quench the reaction. The tube reactor is taken out from the ice water and dried. The end caps and plugs are then removed, and the pretreated substrate is pushed out with a piston-like metal rod and then filtered to separate the liquid from the solids for analysis (*28, 27*). Batch tube pretreatments are run at least in duplicate so that the solids and liquid from one of the tubes can be used for analysis of sugar recovery and composition changes of the solid substrate during pretreatment and the pretreated solids from the other tube are used to determine the effect of pretreatment on cellulose digestibility (**Note 1**).

3.2. Mixed Batch Reactors

Pretreatment of much larger amounts of biomass requires that the contents be mixed to maintain uniform temperatures. For example, a 1-l cylindrical reactor made of Carpenter-20 or Hastelloy C can be employed with a 3.5-in.-diameter helical impeller on a two-piece shaft driven by an adjustable speed DC motor drive (*29*). As shown in **Fig .2**, the agitator motor is attached to the reactor, and the combined systems suspended from a chain hoist mounted to a wall crane. Biomass is loaded into the reactor after being presoaked in the target concentration of dilute sulfuric acid solution at 5% solids (w/w) at room temperature for at least 4 h. A K-type thermocouple and a 1/8-in.

Fig. 2. Mixed Parr reactor pretreatment and fluidized sand bath system.

stainless steel thermocouple probe are inserted through a port in the vessel head to monitor the reactor temperature. The reactor is then closed per manufacturer recommendations, and the impeller is set to the target agitation speed. The reactor is lowered into a 320°C sand bath to the bottom of the reactor head flange to rapidly heat up the biomass to the target temperature in about 2 min. As the target temperature is approached, the reactor is raised out of the sand bath until the bottom is about 1–2 cm above the sand surface. The position of the reactor is then adjusted manually to maintain the temperature within ±2°C of the target temperature. After completion of the target reaction time, the vessel is hoisted out of the sand bath and lowered into a 19-l poly bucket filled with water at room temperature to quench the reaction. The pretreated contents are filtered through a Buchner funnel to separate the liquid from the solids. Room-temperature deionized water is then used to wash the solid cake until the filtrate pH is above 6 (typically about 1 l water is needed). The solid and liquid fractions are then analyzed (**Fig. 2**) (**Note 2**).

3.3. Flow–Through Tubular Reactors

As discussed above, flow of water through a solid bed provides interesting information about the release patterns of hemicellulose, lignin, cellulose, and other components in the biomass ***(24, 30)***. Two small reactor designs have been employed to keep the cost of the high-pressure pump reasonable: a small tubular reactor (½ in. ID × 1.84 in. length) and a larger tubular reactor (½ in. ID × 6 in. length) with an internal volume of 14.3 ml. However, larger units can be constructed if the higher pressure drop can be handled safely and the pump capacity is sufficient. The reactors can be made from Hastelloy C276 tubing for use with dilute acid or from 316 stainless steel for use with just water. Two gasket filters (316 stainless steel, average pore size 5 μm) cover both ends of the reactors. A 316 stainless steel tube (0.25 in. OD × 0.35 in. wall thickness × 50 in. length) is used to preheat water to the desired pretreatment temperature before entering the reactor. Another 316 stainless steel tube (0.25 in. OD × 0.35 in. wall thickness × 50 in. length) is attached to the reactor outlet to cool the effluent.

The flow-through system is illustrated in **Fig. 3**. A high-pressure pump capable of a flow rate ranging from 0 to 40 ml/min is connected to the flow-through reactor to deliver water. A pressure gauge and a backpressure regulator are used to monitor the pressure to the reactor. A 0.25-in. stainless steel thermocouple monitors the temperature at the outlet of the reactor. Biomass substrate is loaded into the flow-through reactor, which is then connected to the inlet and outlet tubing. Water alone or containing dilute sulfuric acid at room temperature is pumped through the reactor for a few minutes to purge out air until the pressure in the reactor increases to the set pressure of 350–400 psig. Flow is then stopped to completely wet the reactor contents, and the reactor

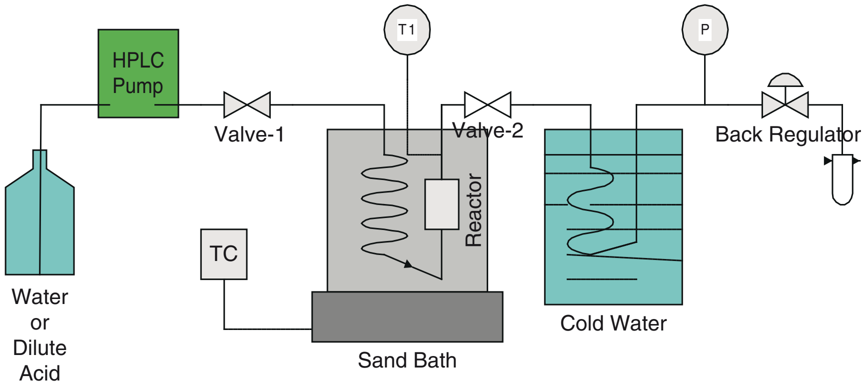

Fig. 3. Flow-through pretreatment reactor system.

and preheating coil are submerged in a 4-kW fluidized sand bath at 100°C for 2 min. Next, the reactor and the preheating coil are quickly moved to a second sand bath set at the target reaction temperature, and liquid is pumped through the device at a set flow rate until the end of desired reaction time. The time of transfer to the second sand bath is defined as zero. Upon reaching the desired reaction time, the reactor and preheating coil are transferred to an ice water bath to quench the reaction. The liquid hydrolyzate leaving the reactor is collected for analysis and measurement of its pH. The contents left in the reactor at the completion of the run are transferred to a filter and washed with a sufficient amount of water for its pH to be greater than 6.

Samples of the liquid hydrolyzate are dried at 60°C in a vacuum dryer until weight loss ceased, to determine the solids content of the liquid fraction. The sugars in the liquid hydrolyzate are analyzed using an HPLC equipped with a refractive index detector. The collected solid residue in the reactor is dried at 105°C, weighed, and then used for analysis of Klason lignin, cellulose, hemicellulose, and other fractions. The amount of lignin removal is estimated on the basis of the difference in the Klason lignin content between that in the original biomass substrate and the pretreated solids residue (**Note 3**).

3.4. Steam-Heated Reactors

Solids concentrations of about 50% by weight are important to application to commercial conditions, but maintaining uniform temperature throughout the course of pretreatment is critical for meaningful interpretation of the data. Tubular reactors can handle high solids successfully, but tube diameters needs to be less than about ½ in. to provide uniform temperatures, thereby limiting the amount of biomass than can be readily pretreated. Larger amounts of material can be produced in mixed reactors, but the solids content must be less than about 10% by weight so mixing is adequate to ensure uniform temperatures. One of the best ways found to process larger amounts of biomass at high solids concentrations while controlling temperatures is through direct steam heating. Steam heating can provide very rapid heat-up to reaction temperatures of typically between 140 and 260°C (37–260 psig), and the temperature can be controlled well by appropriate selection of the steam pressure. Furthermore, rapid release of the pressure at the completion of the run almost instantly drops the temperature to 100°C, terminating the reaction.The term "steam gun," as frequently used to describe this approach, arises from the rapid pressure release when the contents are discharged into a receiver vessel. Residence times can span a range from less than 1 min to 4 h or more.

A number of steam gun reactors have been used to conduct larger batch steam explosion or steam expansion pretreatment with catalysts (e.g., dilute acid, SO_2) or without them *(31–34)*. We recently installed the steam gun, shown in **Fig. 4**, in our

Fig. 4. Steam gun pretreatment reactor system.

laboratory powered by an electric boiler that is rated at 75 kW and can generate up to 800 psig. The steam gun is a cylinder of 4 in. nominal inside diameter and 24 in. length and is constructed of Hastelloy C to handle dilute sulfuric acid if needed. The bottom portion of the unit tapers down gradually to a ¾ in. discharge line so that steam will be distributed evenly throughout the biomass and dead spaces will not be formed in corners at the bottom. A discharge valve is attached to the bottom as close to the reactor body as possible to minimize accumulation of biomass in the dead space above the valve and can be actuated remotely by an electrical device. A ½-in. steam injection line enters the side of the discharge line above and as close to the discharge valve as possible to minimize the dead volume between the steam entrance position and the top of the valve. The vessel is jacketed along its entire length including the bottom of the reactor so that steam can be applied to the internal vessel walls through the jacket, which minimizes heat losses that result in excessive condensation and dilution of the sugars released. The outside of the vessel is heavily insulated to minimize heat losses, but is removable for maintenance. A valve is attached to the port on the top of the steam gun to allow biomass to be readily added to the vessel, and a thermocouple is run through the top into the middle of the interior. One of the bigger challenges with the steam gun is accurate material balance closure because of the loss of steam during discharge, the hang up of some biomass inside the vessel, and difficulties in weighing the discharged material. However, we developed an approach using a drum ring to hold a heavy-gauge polyethylene bag to a drum top attached below the bottom valve to collect material upon discharge and facilitate rapidly closing material balances.

The steam gun above can process about 3–5 lb of dry biomass at a time. Prior to pretreatment, the feedstock is preweighed in batches of 200–350 g dry in plastic containers or bags, and presoaked/preimpregnated with just water or with a catalyst solution (e.g., dilute acid or SO_2) overnight. The steam boiler is turned on following standard procedures, the pressure is set at the target pressure for steam explosion, and the insulated and heat-traced vessel is preheated with steam in the jacket for 2–4 min. to ensure that it is at the target temperature. The preimpregnated feedstock is then loaded into the reactor from the top, and the reactor is sealed. Fresh steam is introduced into the bottom of the vessel, raising the inside temperature to the target value in about 15 seconds. The boiler pressure is usually set at about 10–15 psi above the operating pressure for the steam gun but below the maximum pressure of the relief valve. The steam inlet valve is closed about 10 seconds before the end of the operating time, the discharge valve at the bottom of the steam gun is opened, and the contents are blown into the collecting bag to rapidly bring the temperature to below 100°C and quench the reaction. The discharge valve is opened at least twice to ensure that the vessel is fully depressurized and empty. The reaction time is defined to be from the moment steam is introduced into the reactor until the contents are discharged. The pretreated material is collected, filtered, and washed with water, and the hydrolyzate is sampled for further analysis. The solids are stored at –20°C (**Note 4**).

3.5. Conclusions

Dilute acid pretreatment is one of the leading pretreatment techniques because of the high yields possible in the coupled operations of pretreatment and enzymatic hydrolysis, while autohydrolysis is often favored because of its long use and ease of application. However, the ability to design these systems in conjunction with enzymatic hydrolysis a priori is limited, and detailed material balance data is vital for the assessment and commercialization of this technique.

4. Notes

1. The small-batch tube reactors provide a low-cost, lab-scale option to optimize pretreatment conditions at high solids loading (up to 30%), and several reactor simulation models have been developed on the basis of mass balance results from tube reactors ***(35, 25)***.
2. A mixed reactor can handle larger amounts of material than tube reactors, but handling high solids concentrations (>10%) is challenging for this type of device ***(29)***.
3. We have successfully employed a flow-through reactor configuration to provide valuable data on the kinetics of biomass deconstruction ***(36, 37)*** and to test the applicability of rate laws. However, although high sugar yields and high lignin removal can be achieved with flow-through systems ***(31, 24)***, no one has yet found a way to employ them commercially.
4. The steam gun reactor can be operated at the high solids concentrations desired commercially, process large amounts of biomass, heat up biomass rapidly, control temperature well, and cool the products quickly. However, these systems are more expensive and present bigger challenges to closing material balances as accurately.

Acknowledgements

We are grateful for support from the Ford Motor Company Chair in Environmental Engineering through the Center for Environmental Research and Technology (CE-CERT) of the Bourns College of Engineering at the University of California at Riverside and for support from the Chemical and Environmental Engineering Department at UCR.

References

1. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., and Lee, Y. Y. (2005) Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover, *Bioresource Technology* 96 (18), 2026–2032
2. Boehm, R. M. (1930) The Masonite process, *Journal of Industrial and Engineering Chemistry* 22, 493–497
3. Heitz, M., Capek-Menard, E., Koeberle, P. G., Gagne, J., Chornet, E., Overend, R. P., Taylor, J. D., and Yu, E. (1991) Fractionation of Populus tremuloides at the pilot plant scale: optimization of steam pretreatment conditions using the STAKE II technology, *Bioresource Technology* 35 (1), 23–32
4. Jollez, P., Chornet, E., and Overend, R. (1994) Steam-aqueous fraction of sugar cane bagasse: an optimization study of process conditions at the pilot plant level, *Advances in Thermochemical Biomass Conversion, [Ed. Rev. Pap. Int. Conf.], 3rd*, 2, 1659–1669
5. Saeman, J. F. (1945) Kinetics of wood saccharification: Hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature, *Industrial Engineering & Chemistry Research* 37, 42–52
6. Harris, J. F., Baker, A. J., Conner, A. H., Jeffries, T. W., Minor, J. L., Petterson, R. C., Scott, R. W., Springer, E. L., Wegner, T. H., and Zerbe, J. I. (1985) General Technical Report No. FPL-45, Forest Products laboratory, Madison
7. Knappert, D. R., Grethlein, H. E., and Converse, A. O. (1980) Partial acid hydrolysis of cellulosic materials as a pretreatment for enzymatic hydrolysis, *Biotechnology and Bioengineering Symposium* 22, 1449–1463
8. Grethlein, H. E. (1980) Pretreating cellulosic substrates and producing sugar therefrom. US 4237226
9. Yang, B., Gray, M. C., Liu, C., Lloyd, T. A., Stuhler, S. L., Converse, A. O., and Wyman, C. E. (2004) Unconventional relationships for hemicellulose hydrolysis and subsequent cellulose digestion. ACS Symposium Series 889 (Lignocellulose Biodegradation), 100–125
10. Lee, Y. Y., Iyer, P., and Torget, R. W. (1999) Dilute-acid hydrolysis of lignocellulosic biomass, *Advances in Biochemical Engineering/Biotechnology* 65 (Recent Progress in Bioconversion of Lignocellulosics), 93–115
11. Lightner, G. E. (1999) Process for production of solid glucose from acid hydrolysis of biomass. US 5868851
12. Lightner, G. E. (2001) Method to produce fermentable sugars from a lignocellulose material. US 6258175
13. Torget, R. W. (2000) Aqueous fractionation of biomass based on novel carbohydrate hydrolysis kinetics. WO 0061276
14. Von Sivers, M. and Zacchi, G. (1995) A techno-economical comparison of three processes for the production of ethanol from pine, *Bioresource Technology* 51, 43–52
15. Wright, J. D. and d'Agincourt, C. G. (1984) Report No. DE-ACO2–83CH10093
16. Wyman, C. E. (1996) Progress on ethanol production from lignocellulosic biomass, *Biomass Energy Environ., Proc. Eur. Bioenergy Conf., 9th* 1, 356–361
17. Bobleter, O., Bonn, G., and Concin, R. (1983) Hydrothermolysis of biomass – production of raw material for alcohol fermentation and other motor fuels, *Altern. Energy Sources* 3 (3), 323–332
18. Bobleter, O., Grif, M., and Huber, C., DE 4401992, (1994) Thermal hydrolysis of plant materials with water and alkaline solutions
19. Bonn, G., Oefner, P. J., and Bobleter, O. (1988) Determination of organic acids formed during hydrothermal and organosolv degradation of lignocellulosic biomass, *Fresenius' Zeitschrift fuer Analytische Chemie* 331 (1), 46–50

20. Hoermeyer, H. F., Schwald, W., Bonn, G., and Bobleter, O. (1988) Hydrothermolysis of birchwood as pretreatment for enzymic saccharification, *Holzforschung* 42 (2), 95–98
21. Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal, M. J., and Lynd, L. R. (2001) A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, *Bioresource Technology* 81 (1), 33–44
22. Torget, R., Hatzis, C., Hayward, T. K., Hsu, T.-A., and Philippidis, G. P. (1996) Optimization of reverse-flow, two-temperature, dilute-acid pretreatment to enhance biomass conversion to ethanol, *Applied Biochemistry and Biotechnology* 57/58, 85–101
23. van Walsum, G. P., Allen, S. G., Spencer, M. J., Laser, M. S., Antal, M. J., Jr., and Lynd, L. R. (1996) Conversion of lignocellulosics pretreated with liquid hot water to ethanol, *Applied Biochemistry and Biotechnology* 57/58 (Seventeenth Symposium on Biotechnology for Fuels and Chemicals, 1995), 157–170
24. Yang, B. and Wyman, C. E. (2004) Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose, *Biotechnology and Bioengineering* 86 (1), 88–95
25. Stuhler, S. L. and Wyman, C. E. (2003) Estimation of temperature transients for biomass pretreatment in tubular batch reactors and impact on xylan hydrolysis kinetics, *Applied Biochemistry and Biotechnology* 105–108, 101–114
26. Jacobsen, S. E. and Wyman, C. E. (2001) Heat transfer considerations in design of a batch tube reactor for biomass hydrolysis, *Applied Biochemistry and Biotechnology* 91–93 (Symposium on Biotechnology for Fuels and Chemicals, 2000), 377–386
27. Lloyd, T. and Wyman, C. E. (2003) Application of a depolymerization model for predicting thermochemical hydrolysis of hemicellulose, *Applied Biochemistry and Biotechnology* 105–108, 53–67
28. Stuhler, S. L. (2002) *Effects of solids concentration, acetylation, and transient heat transfer on uncatalyzed batch pretreatment of corn stover*, MS Thesis, Hanover, NH
29. Brennan, M. A. (2003) *Predicting performance of batch, flowthrough, and mixed batch hemicellulose hydrolysis by coupled mass transfer and reaction models*, MS, Dartmouth College, Hanover, NH
30. Liu, C. and Wyman, C. E. (2003) The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover, *Industrial & Engineering Chemistry Research* 42 (21), 5409–5416
31. Foody, F. (1980) Final Report No. DE-AX-02–79-ETZ3050. Prepared for U.S. Department of Energy Fuels from Biomass Program
32. Dekker, R. F. H. and Wallis, A. F. A. (1984) Enzymic saccharification of sugarcane bagasse pretreated by autohydrolysis-steam explosion, *Biotechnology and Bioengineering* 25, 3027–3048
33. Brownell, H. H. and Saddler, J. N. (1987) Steam-explosion pretreatment for enzymatic hydrolysis, *Biotechnology and Bioengineering*. 14, 55–68
34. Overend, R. P. and Chornet, E. (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments, *Philosophical Transactions of the Royal Society of London Series A-Mathematical Physical and Engineering Sciences* 321 (1561), 523–536
35. Lloyd Todd, A. (2004) *Fragmentation of hemicellulose during pretreatment: the predicted effect of sulfuric acid on sugar release*, Dartmouth College, Hanover, NH
36. Gray, M. C., Converse, A. O., and Wyman, C. E. (2007) Solubilities of oligomer mixtures produced by the hydrolysis of xylans and corn stover in water at 180 Degree C, *Industrial & Engineering Chemistry Research* 46 (8), 2383–2391
37. Yang, B. and Wyman, C. E. (2008) Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water, *Bioresource Technology* 99 (13), 5756–5762